Metabolic and Hemodynamic Effects of CO₂ Pneumoperitoneum in a Controlled Hemorrhage Model

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Background: Intracavity infusion of fibrin sealant-based agents, as a novel modality to control internal bleeding, is associated with an increase of pneumoperitoneum (PP) pressure. The safe limit of such increase has not been well defined in hypovolemic subjects. The purpose of this study was to evaluate the hemodynamic and metabolic effects of increasing PP pressure and to define the limits of carbon dioxide (CO_2) insufflation in a controlled hemorrhage rat model.

Methods: Ninety male rats (474 ± 6 g, 37° ± 1°C) were anesthetized, and mechanically ventilated. Animals were randomly distributed among 14 groups (n = 6–8) with an increasing amount of blood loss (0, 10, 15, and 17.5 mL/kg) and 15 minutes of CO_2 insufflation at 0, 5, 10, and 15 mm Hg starting 15 minutes after hemorrhage, followed by desufflation. Mean arterial pressure (MAP), heart rate, and survival were recorded and arterial and venous blood samples were collected at baseline, at 15 minutes after hemorrhage, after insufflation, and after desufflation procedures to determine arterial blood gases and lactic acid levels.

Results: In nonhemorrhaged animals, increasing PP pressure up to 15 mm Hg produced only transient changes in MAP and no increase in lactate level. A moderate hemorrhage (10 mL/kg) limited the safe abdominal pressure to 10 mm Hg with metabolic changes that were restored 15 minutes after desufflation. Higher PP pressure (15 mm Hg) at this hemorrhage level produced a significant decline in MAP (42%, $p < 0.001$) and progressive metabolic acidosis with a 2.1-fold increase ($p < 0.01$) in lactate level. The more severe hemorrhage (15 mL/kg) further reduced the limits of PP pressure such that 10 and 15 mm Hg resulted in a progressive decline of blood pressures (52% and 54%, respectively; $p < 0.001$) and severe metabolic acidosis as manifested by 3.3- and 3.1-fold rises in lactate levels, respectively. In the most severe hemorrhaged animals (17.5 mL/kg), the 50% mortality was primarily determined by the severity of the blood loss and the additional PP at 5 mm Hg had no significant impact.

Conclusion: The safe limit of PP pressurization with CO_2 is dependent on the amount of blood loss. In this mechanically ventilated rat model, increasing the amount of blood loss from 0 to 15 mL/kg reduces the tolerable level of abdominal insufflation pressure from 15 mm Hg to 5 mm Hg. A 5-mm Hg PP pressure appears safe even in the most severely hemorrhaged animals.

Key Words: Pneumoperitoneum, Hemorrhage, Abdominal compartment syndrome.

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I n the emergency trauma setting, laparoscopy may be a useful tool for rapid and minimally invasive assessment of abdominal injuries. However, the establishment of a safe and stable visual field is a critical step in laparoscopic surgery that usually requires the insufflation of the peritoneal cavity with an inert gas, most commonly carbon dioxide (CO_2), to an intraperitoneal pressure of 12 to 15 mm Hg. Such pressures have resulted in significant systemic and pulmonary effects in hemodynamically compromised patients.[1,2] Compression of the major vasculature and serosal capillary beds causes alterations of regional blood flow that may result in significant metabolic acidosis and potentially lead to an abdominal compartment-like syndrome. Pressure on the inferior aspect of the diaphragm restricts ventilation and increases airways pressures leading to hypoxemia and respiratory acidosis. Transperitoneal absorption of CO_2 may also contribute to the development of acidosis.[3] In relatively healthy patients with normal blood pressures, the intra-abdominal pressure exerted during laparoscopy is well tolerated and hypercarbia secondary to a CO_2 pneumoperitoneum (PP) is easily resolved by the increase of the alveolar ventilation and an increase in end-tidal CO_2 tension. However, in acutely injured patients that are hypovolemic or hypotensive, the hemodynamic effects of PP could become irreversible, and the increased CO_2 load can result in clinically profound hypercapnia, acidemia, and death.[4]

A new technique that uses the fibrin sealant (FS) components as foam is being developed to control the internal bleeding caused by parenchymal injuries. The reconstituted FS foam is rapidly sprayed into the abdominal cavity and allowed to clot. In a proof-of-concept study, the spongy fibrin clot adhered to injured liver tissue and stopped the

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hemorrhage.[5] A significant expansion of the FS foam matrix occurs on mixing that is associated with the rapid generation and release of CO_2 gas. The CO_2 gas that drives the product may result in a high PP pressure. To establish a tolerable pressure limit for expanding FS foam, it was essential to determine the safe levels of PP under varying degrees of acute hemorrhagic shock. We performed this study to determine the maximum PP pressure that is safely tolerated by hypotensive animals with different degrees of blood loss.

MATERIALS AND METHODS

Because this initial study was designed to cover numerous conditions and therefore required testing a large number of the animals, the rat was chosen as the animal model. The use of rodents in laparoscopic surgery has been reported,[6,7] and the effect of CO_2 pneumoperitoneum was shown in normal rats.[8,9]

A total of 90 male Sprague-Dawley rats (Zivic Miller, Porterville, PA), 120 to 180 days old, weighing between 400 and 500 g (474 ± 6 g), were used in this study. Animals were held at the animal facility of the American Red Cross Holland Laboratory that is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. They received husbandry care in accordance with the Animal Welfare Act and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals, 1996.* This study was approved by the Institutional Animal Care and Use Committee of the Holland Laboratory.

Rats were premedicated with subcutaneous atropine sulfate (0.05 mg/kg) and buprenorphine (0.6 mg/kg) injections, and anesthetized with a combination of ketamine HCl (100 mg/mL) and acepromazine (10 mg/mL) anesthetics (9:1 ratio) at the dose of 100 mg/kg, injected intramuscularly. Diazepam (1.0 mg/kg) was also administered intraperitoneally to avoid animal resistance against the positive-pressure mechanical ventilation. An additional dosage of the ketamine/acepromazine mixture at one fourth the initial dose was given at approximately 1 hour into the procedure. Once the rat was anesthetized, its ventral neck region was shaved and disinfected with alcohol swabs. The rat was secured on a small operating board in a supine position. A small lubricated thermoprobe attached to a digital thermometer (Physitemp, Clifton, NJ) was placed rectally to monitor the animal's core body temperature. The rat's body temperature was maintained at 37°C during the operation by the use of an electric heating pad placed underneath the operating board. A 3-cm midventral incision was made, and the right external carotid artery was exposed and cannulated with polyethylene tubing (PE-50). This line was prefilled with heparinized saline and attached to a pressure transducer (Micro-Med, Louisville, KY) for monitoring heart rate, blood pressure (systolic, diastolic, and mean arterial), and drawing blood samples for blood gas analysis. Blood pressure and heart rate data were collected by the Digi-Med Blood Pressure Analyzer (Model 190, Micro-Med) at 5-second intervals, averaged over a

1-minute period, and recorded in an ASCII file by the computer. The femoral vein was also cannulated with similar tubing to obtain venous blood samples for lactic acid determination. The trachea was incised between cartilage rings, and a short plastic tube (2 mm outside diameter) was inserted and attached to a mechanical ventilator (Harvard Apparatus, Holliston, MA). Rats were ventilated with filtered air at the rates of 60 to 70 breaths/min and tidal volumes of 2.5 to 3 mL under positive pressure. The respiration rate was adjusted to measure the normal arterial P_{CO_2} level (~40 mm Hg) before the experiment was started. Arterial P_{O_2}, P_{CO_2}, pH, and bicarbonate were determined immediately after each blood draw (0.2 mL) using a portable blood gas analysis unit (i-STAT, SDI Sensor Devices, Inc., Waukesna, WI). The venous lactic acid levels were measured using a calorimetric assay kit obtained from Sigma Chemical Co. (St. Louis, MO). The venous blood samples (0.2–0.3 mL) were immediately mixed with an appropriate anticoagulant (potassium oxalate and sodium fluoride), centrifuged (5 minutes at 5,000 revolutions per minute), and the plasma supernatant stored at −20°C until assayed.

The blood pressure recording was started once the rat was instrumented and the ventilation rate was optimized. The animal was included in the study only if the mean arterial pressures of the rat remained above 85 mm Hg for the next 10 minutes. The first arterial and venous blood samples (baselines) were drawn 10 minutes after the data recording started. Depending on the hemorrhage group, the controlled bleeding was then initiated by drawing 10, 15, or 17.5 mL/kg body weight blood from the carotid artery. Blood draw was at a constant rate (3 mL/min) and took from 1.3 to 3 minutes to complete from each rat. The 3 mL/min rate produced a rapid drop in mean arterial pressure (MAP), replicating the drop in uncontrolled hemorrhage. Animals were kept warm and allowed to recover. Fifteen minutes later, when the blood pressure stabilized, the second group of blood samples was drawn. Two polytetrafluoroethylene (Teflon) intravenous catheters (22 gauge) were then inserted into the peritoneal cavity on each side of the abdomen. A 60-mL syringe filled with CO_2 was attached to one of the catheters and used for the abdominal insufflation and the other one was connected to a digital manometer (475-1 series, Dwyer Instruments, Inc., Michigan City, IN) to monitor the PP pressure. A total of 180 to 240 mL CO_2 was injected intraperitoneally to generate pressures equal to 5 to 15 mm Hg. The pressure was maintained for 15 minutes, and a third set of blood samples was drawn. The pressure was then released as the syringe and manometer were disconnected from the catheters. The abdomen was also gently compressed to force out any residual CO_2. The fourth blood samples were drawn 15 minutes after the desufflation. In some cases, additional blood samples were taken 10 minutes later to follow up the recovery trend of the animals. The volume of blood drawn for sampling was replaced with an equal volume of normal saline. A schematic

Experiment Protocol
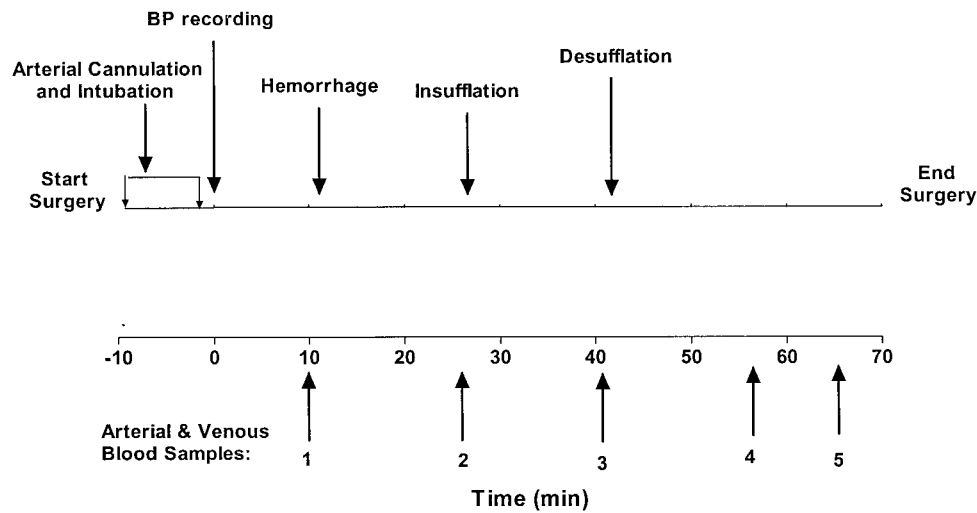
Fig. 1. *Schematic representation of the experiment protocol. The times at which the animals were instrumented, hemorrhaged, insufflated, desufflated, and blood samples were taken are displayed.*

representation of the experiment protocol and the times that blood samples were drawn is shown in Figure 1.

Experimental Groups

Rats were randomly distributed among 14 experimental groups, with six to eight animals in each group. The complete list of the groups, which includes various degrees of hemorrhage and PP pressures, is presented in Table 1. Since our preliminary experiments showed that the withdrawal of 17.5 mL/kg blood at the rate of 3 mL/min alone produces nearly 50% mortality in the rats, the insufflation pressure above 5 mm Hg in this group appeared excessive and was eliminated from this study.

Data are expressed as mean $\pm$ SEM. The mean arterial pressures, blood gases, pH, and lactate levels at different intervals were compared by one-way analysis of variance test. Comparisons between the groups were performed using a Newman-Keuls test. Significance was assigned at a greater than 95% confidence level ($p < 0.05$).

RESULTS

The overall finding of this study is simplified on the basis of the safe metabolic and hemodynamic changes under various experimental conditions and presented in Table 2. The safe level of PP, in this study, is defined as the insufflation pressure that has minor to mild effects on the circulatory system, allowing the low blood pressure to remain stable and the moderate hypercapnia and acidemia developed during insufflation to be reversed once the PP pressure is released.

Using the anesthetic regimen described in the study, the baseline mean arterial pressures recorded for 10 minutes at the start of the experiments ranged from 86 $\pm$ 5 mm Hg to 105 $\pm$ 7 mm Hg. There were no significant differences among the 14 groups. The relatively acute blood draw (3 mL/min) from the control animals in the amounts of 10, 15, and 17.5 mL/kg produced an immediate and sharp decline in the mean arterial pressures. The pressures gradually rose and stabilized 10 minutes after blood draw at the levels significantly below the normal (70 $\pm$ 10, 55 $\pm$ 6, and 35 $\pm$ 4 mm Hg, respectively; $p < 0.001$) (Fig. 2). The final pressures reached in the 17.5-mL/kg group were also significantly less than the 10- or 15-mL/kg blood draw animals ($p < 0.01$).

In normovolemic control animals, increasing levels of PP up to 15 mm Hg with CO₂ appeared to be harmless, producing only a slight reduction in MAPs (Fig. 3). A transient rise of blood pressure was observed consistently after decompres-
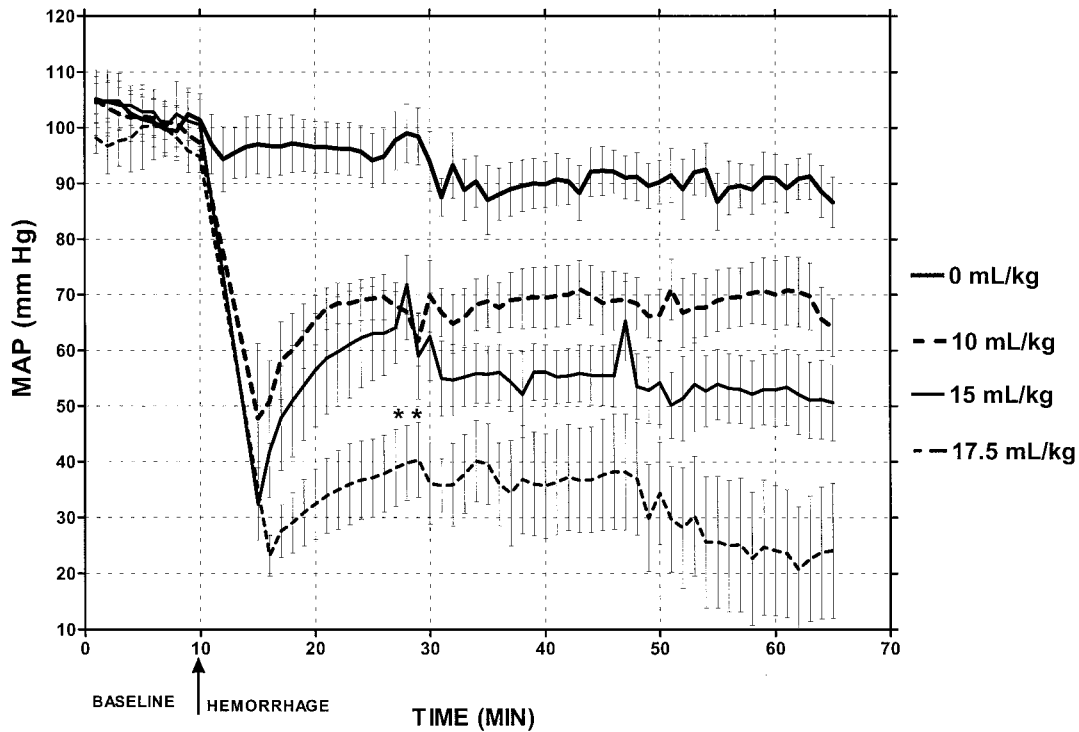
Table 1 Experimental Groups

| Blood Draw (mL/kg)	Pneumoperitoneum Pressure (mm Hg)			
	0	5	10	15
0	8	6	6	6
10	7	6	7	6
15	6	6	8	6
17.5	6	6	—	—

Values refer to the number of animals tested in each group.

Table 2 Summary of the Results

| Blood Draw (mL/kg)	Pneumoperitoneum Pressure (mm Hg)			
	0	5	10	15
0	Safe	Safe	Safe	Safe
10	Safe	Safe	Safe?	Not safe
15	Safe	Safe	Not safe	Not safe
17.5	Not safe	Not safe	—	—

Fig. 2. *The mean arterial pressures of rats after 0, 10, 15, and 17.5 mL/kg controlled arterial hemorrhage. The data were averaged for each group and expressed as mean ± SEM. The various hemorrhage protocols produced an immediate and sharp decline in blood pressures that was only partially restored. The stabilized pressure reached 15 minutes after the hemorrhage was significantly less in the 17.5-mL/kg group than the other hypotensive groups (p < 0.01).*
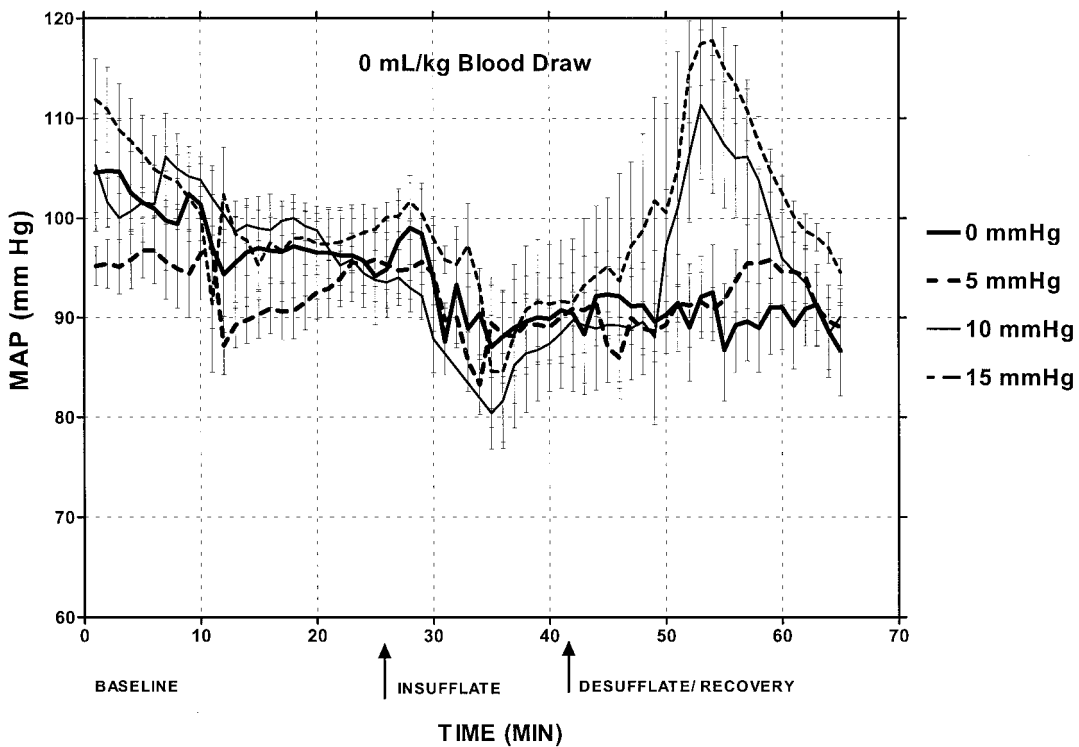
Fig. 3. *The averaged mean arterial pressures of the rats insufflated with CO_2 at 0, 5, 10, and 15 mm Hg for 15 minutes. The transient pressure rise seen in the 10- and 15-mm Hg groups may be the result of an increase in venous return that occurred after desufflation.*
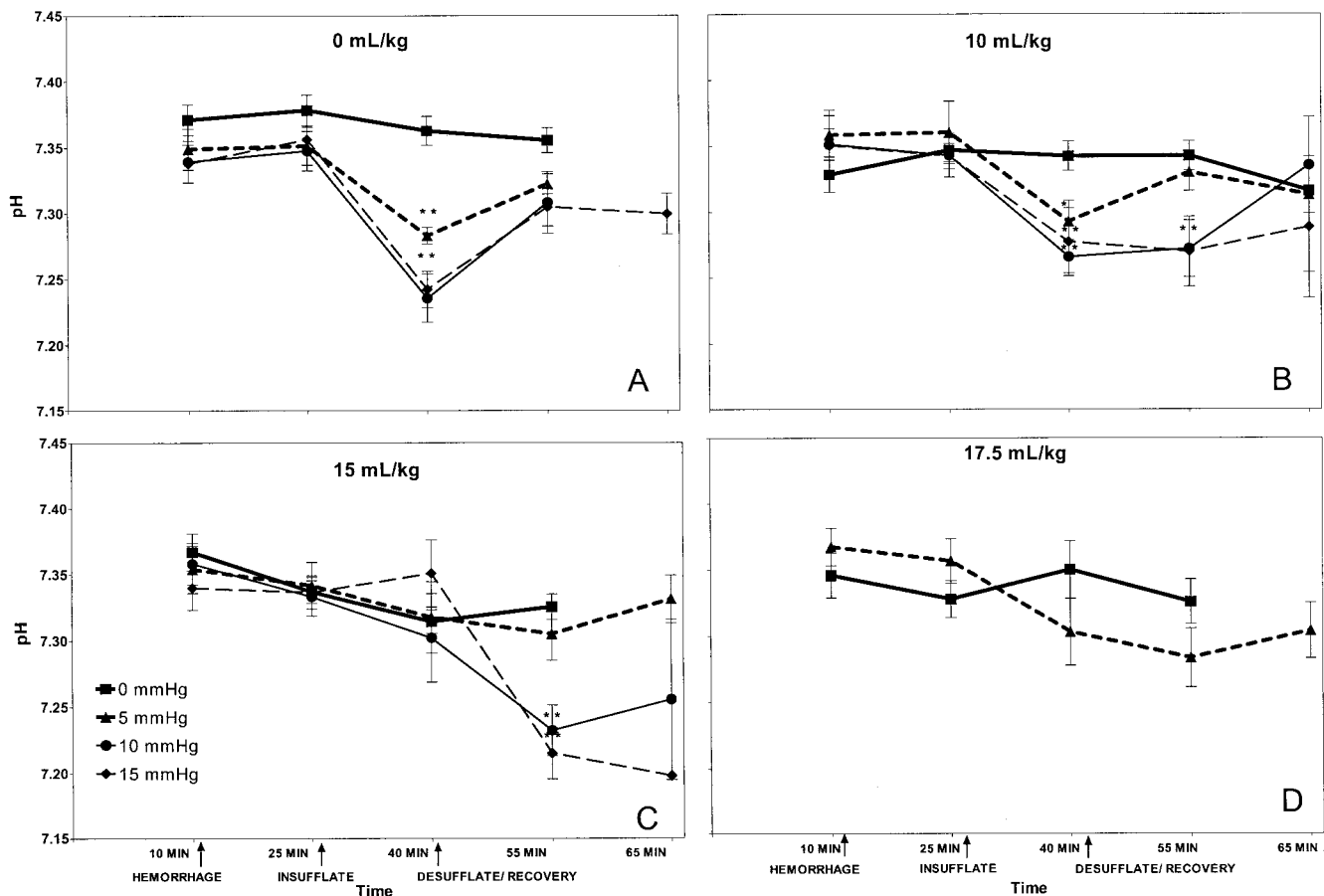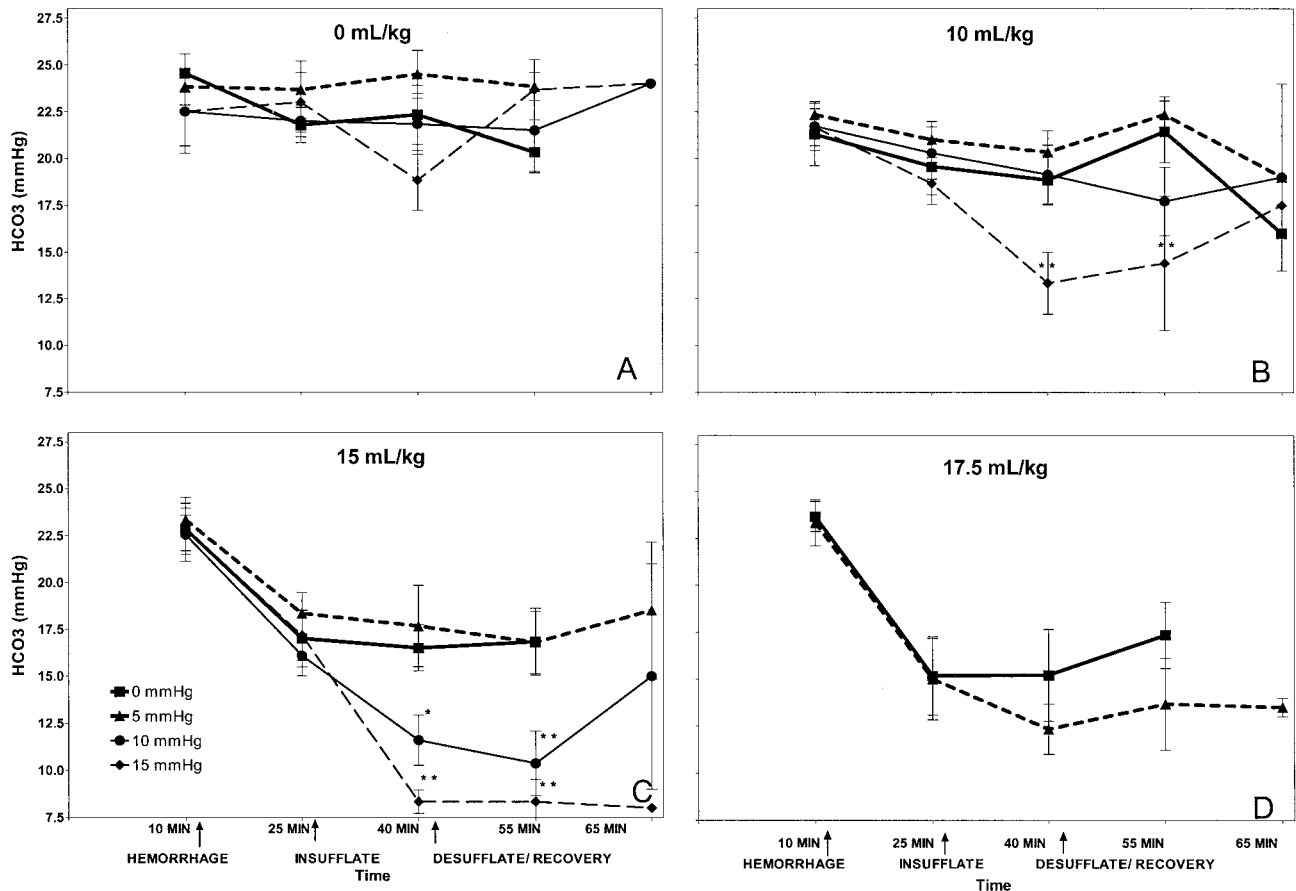
Fig. 4. *The pH of the arterial blood samples taken at 15-minute intervals during the experiment. The data were averaged for each group and expressed as mean ± SEM. In normovolemic animals, CO$_2$ insufflation at 10 to 15 mm Hg produced moderate acidosis that was reversed within 15 minutes after desufflation (A). The same levels of PP in the moderately bled rats (10 mL/kg) resulted in acidosis conditions that required a longer time to normalize (B). In the rat groups that were bled at 15 mL/kg, the most severe acidosis was measured 15 minutes after the release of the insufflation pressures (for the 10- and 15-mm Hg groups [C]), suggesting a possible obstruction of venous return during the higher insufflation pressures. No significant change in the blood pH was measured in the most severe hypovolemic animals (bled 17.5 mL/kg), which were insufflated at 5 mm Hg (D).*

sion in the animals that were insufflated at the level of 10 to 15 mm Hg. The blood gas analysis of this group revealed significant reduction in pH ($p < 0.05$) that normalized 15 minutes after desufflation (Fig. 4A). No other significant change was found in HCO$_3$, Pco$_2$, and Po$_2$ levels during insufflation among these groups as compared with controls (panel *A* of Figs. 5–7). The lactic acid levels remained relatively unchanged throughout the procedure (Fig. 8A).

In the moderately hemorrhaged animals (10 mL/kg), the safe abdominal pressure was 10 mm Hg. This produced a slight reduction in mean arterial pressure during insufflation and a compensatory rise after decompression (Fig. 9). Although the pH dropped in all the insufflated animals (Fig. 4B), other blood gas measurements and lactic acid levels did not change significantly. Insufflation pressure of 15 mm Hg, however, produced a progressive and significant decline in the mean arterial pressure (42%, $p < 0.001$) that was only partially restored when the pressure was released (Fig. 9).

The metabolic acidosis produced during insufflation and desufflation was only partially neutralized by blood buffering and respiratory compensation. This resulted in a significant reduction of HCO$_3$ and Pco$_2$ levels (panel *B* of Figs. 5 and 6). An increase of Po$_2$ was also noted in this group, which was reversed ~20 minutes after desufflation (Fig. 7B).

In the more severely hemorrhaged rats (15 mL/kg), the safe limit of PP pressure was only 5 mm Hg. This pressure was well tolerated by the hypotensive rats, causing no further reduction in their MAP (Fig. 10). The drop in arterial pH and bicarbonate and the rise of lactic acid levels in this group were similar to the control animals (hemorrhaged but not insufflated; panel *C* of Figs. 4, 5, and 8). Higher insufflation pressures, 10 or 15 mm Hg, however, produced a significant and progressive decline in mean arterial pressures (52% and 54%, respectively; $p < 0.001$). The hypotension did not reverse after desufflation (Fig. 10). A marked reduction in pH was also observed after desufflation ($p < 0.01$) that was

Fig. 5. *The HCO$_3$ levels of the arterial blood samples taken at 15-minute intervals during the experiment. In normovolemic rats, the different CO$_2$ insufflation protocols did not change the levels of HCO$_3$ considerably (A). The rats that were bled at 10 mL/kg showed a significant reduction in their HCO$_3$ levels when they were insufflated to 15 mm Hg (B). In the rat groups that were bled at 15 mL/kg, the CO$_2$ insufflation at 10 and 15 mm Hg produced a greater depletion of bicarbonate that persisted even after desufflation (C). A pronounced depletion of bicarbonate also occurred in the rats that were bled the most severely (17.5 mL/kg). This change was only slightly exaggerated when the additional 5 mm Hg insufflation pressure was imposed (D).*

preceded by a significant decline in bicarbonate and P$_{CO_2}$ levels ($p < 0.01$; panel *C* of Figs. 4–6). The progressively severe metabolic acidosis developed during the experiment was manifested by a 3.1- to 3.3-fold accumulation of lactic acid in the blood after the desufflation (Fig. 8*C*). Furthermore, during the insufflation, the arterial P$_{O_2}$ rose significantly above the normal levels for all groups, although ventilation was kept constant throughout the experiment. This trend reversed after desufflation with a recovery of circulation for the lower pressure groups (5 and 10 mm Hg), but persisted in the animals that were pressurized up to 15 mm Hg (Fig. 7*C*). A noticeable bradycardia was also measured in the hypotensive animals with 10 or 15 mm Hg PP pressure. The change in the heart rate was partly reversed but still remained below normal level after desufflation (data not shown).

In the most severe case of hemorrhage (17.5 mL/kg), where up to 25% of circulating blood volume was acutely removed, a marked reduction in arterial blood pressure was

observed (Fig. 11) that led to death of 50% of control (three of six) and 33% of insufflated animals (two of six). This occurred at various times during the experiment. Abdominal insufflation to 5 mm Hg pressure had no additional impact on the blood pressure responses, arterial blood gases, and lactate levels of the surviving animals. These parameters were not different from those of animals that were severely hemorrhaged only (panel *D* of Figs. 5–8 and 11).

DISCUSSION

It is well known from experimental and clinical studies that increasing intra-abdominal pressure (IAP) by CO$_2$ pneumoperitoneum leads to a condition similar to abdominal compartment syndrome, with impaired cardiac, pulmonary, and renal function. The consequences are a significant reduction in cardiac output caused by a diminished venous return, increased peripheral resistance, or both;[10–12] hypercarbia and respiratory acidosis that occurs as a consequence of ventilation insufficiency, secondary to the elevation of the dia-
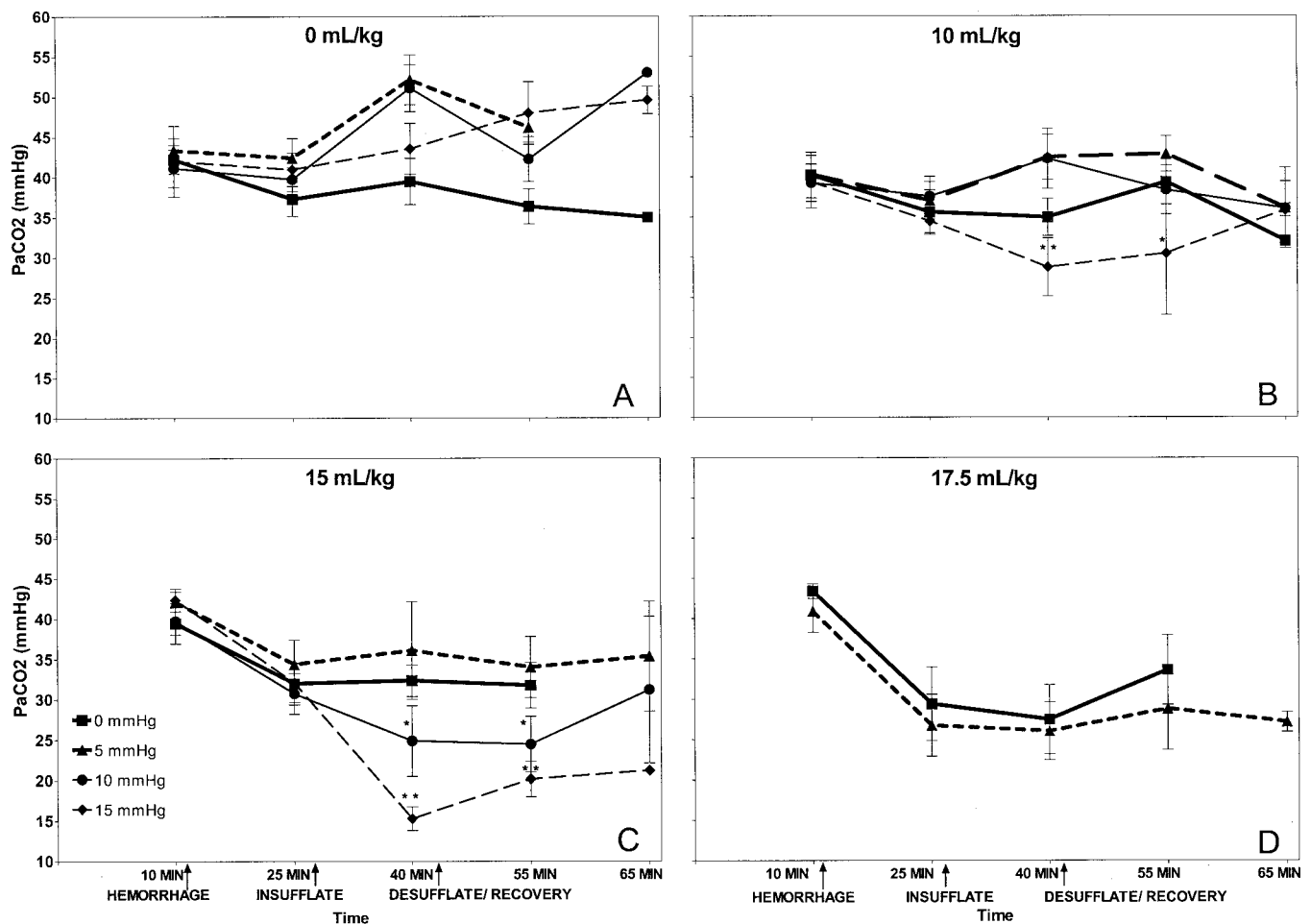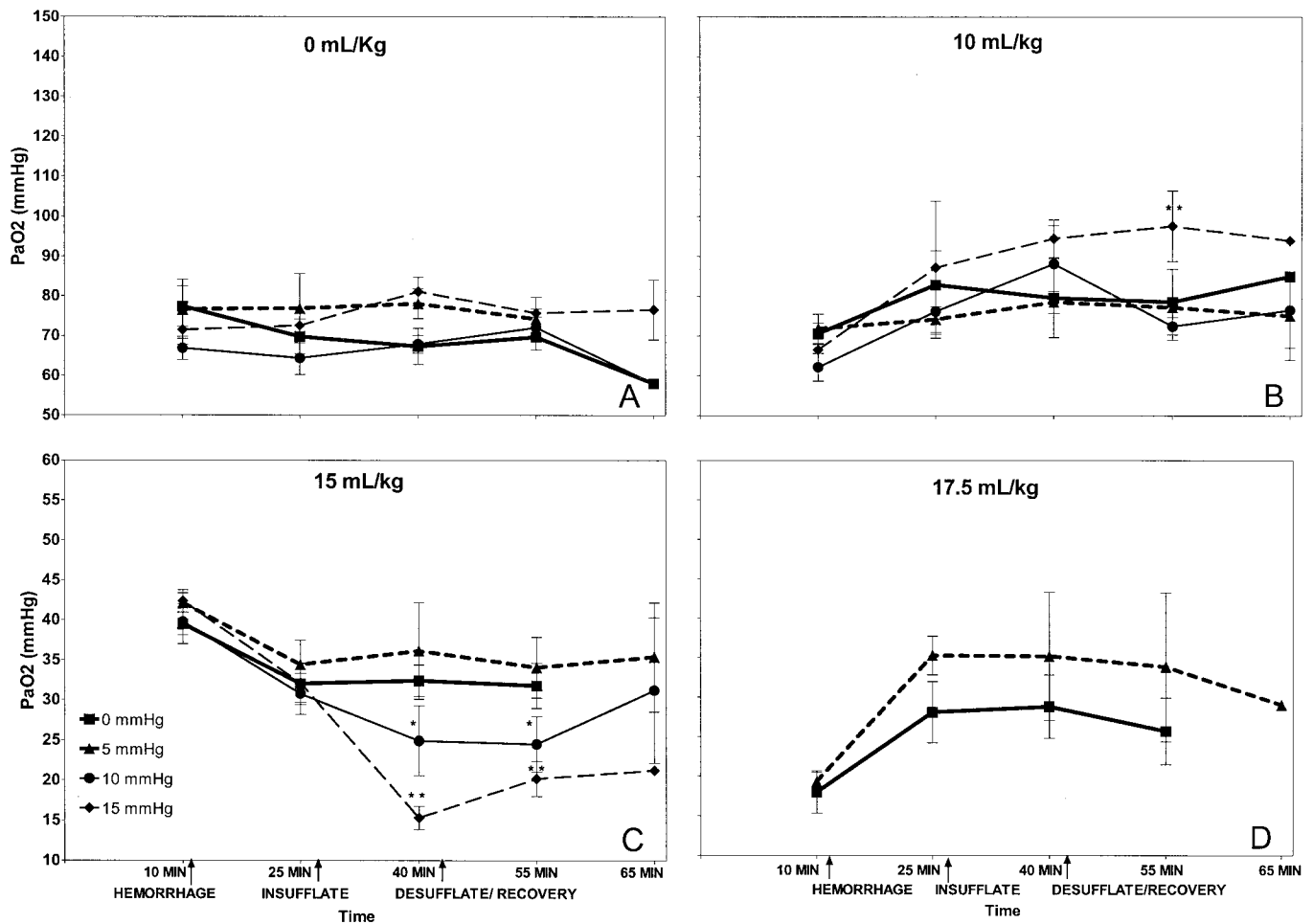
Fig. 6. *The Pco_2 levels of the arterial blood samples taken at 15-minute constant intervals during the experiment. The abdominal insufflation of normotensive rats with CO$_2$ showed no clear trend in the changes that occurred in the Paco_2 levels (A). In moderately hemorrhaged rats (10 mL/kg), a significant reduction in Paco_2 was noted during the insufflation (p < 0.01) that was slowly restored by 25 minutes after desufflation (B). In the more severely hemorrhaged rats (15 mL/kg), the insufflation pressures of 10 and 15 mm Hg were both associated with a marked and persistent decline in Paco_2 levels (C). The large blood draw (17.5 mL/kg) in the last group resulted in a marked reduction in Paco_2 levels with no additional effect of the insufflation procedure (D).*

phragm and the compression of lungs, and partial CO$_2$ absorption from the peritoneum;[3] and oliguria or anuria that may lead to renal failure.[13,14] The decrease in urinary output is related to diminished renal blood flow and the direct compression of the kidney that limits the total cortical blood flow.[15–17] In addition to the kidneys, the blood flow to nearly all the abdominal organs is also reduced.[18] In the case of the liver, a significant decrease in hepatic arterial, portal venous, and microcirculatory blood flow has been reported.[19–21] Acute elevation of IAP is also associated with an increase of intracranial pressure that may cause brain dysfunction or aggravate head injury edema.[22–24]

The decrease in regional blood flow with concomitant reduction in oxygen (O$_2$) transport will decrease O$_2$ availability to the tissues. When the local O$_2$ supply is insufficient to satisfy the requirements of oxidative metabolism, the alternative anaerobic metabolism by the mitochondria results in

a lactate accumulation in the blood that can be an excellent marker for critical hypoperfusion of the tissue.[25] The liberated lactic acid is buffered by bicarbonate, producing CO$_2$ that is expelled by the lungs and results in a decrease of bicarbonate in body fluids. Respiratory compensation also occurs by the increase in ventilatory rate and alveolar ventilation that lowers alveolar and arterial blood Pco_2. The Pco_2 reduction may also occur because of limited production/ diffusion of CO$_2$ from severely hypoperfused tissues.

The safety limits of PP for laparoscopic procedures in trauma patients with severe hemorrhage have not yet been systematically evaluated, although the potential danger to these patients has been recognized.[26] The hemodynamic and metabolic effects of CO$_2$ PP have been studied in hemorrhaged animal models,[27–29] and the findings generally support the concept that acute blood loss exaggerates the deleterious effects of pneumoperitoneum pressure.

Fig. 7. *The Po_2 levels of the arterial blood samples taken at 15-minute intervals during the experiment. The various PP protocols had no measurable effects on Pao_2 levels of the normotensive rats (A). The insufflation pressure of 15 mm Hg in the 10-mL/kg group was associated with a moderate increase of Pao_2 that lasted during the entire experiment (B). In the 15-mL/kg blood draw group, a significant increase (p < 0.01) in arterial blood oxygenation was observed in all insufflated animals. The Pao_2 levels returned to normal after desufflation in the 5- and 10-mm Hg groups but remained high in the 15-mm Hg group (C). An increase in Pao_2 was also noted in the 17.5-mL/kg blood draw groups that was further enhanced in the insufflated animals (5 mm Hg [D]). These changes appear to be in response to excessive blood loss rather than PP in the rats.*

The purpose of this study was to determine systematically the safe levels of CO_2 PP pressure under different hemorrhagic shock conditions. The safe limit was defined as the maximum PP pressure that did not cause circulatory shock or irreversible acidosis in acutely hemorrhaged animals. Since this study was designed to maximize evaluation of many variables, and therefore required a large number of animals to be tested, the rodent species was selected for this investigation.

The blood-draw protocol, which simulated a controlled, unresuscitated hemorrhage, was survivable when up to 15 mL/kg (20% of circulating blood volume) blood was drawn over ~2.5 minutes. Slightly larger blood draws, 17.5 mL/kg, led to a circulatory collapse after the third blood sampling, resulting in 50% mortality in the affected animals. The lactic acid build-up in the surviving animals was effectively buff-

ered and blood pH was maintained at a relatively normal level. This compensation produced significant reductions in bicarbonate and Pco_2 levels in the blood. The circulatory collapse and the metabolic acidosis were the consequences of the hemorrhagic shock; the CO_2 PP in this group had no significant impact on the overall outcome of the experiment. Despite a constant respiratory rate and tidal volume, a respiratory compensation was observed in the animals with high lactate levels. We speculate that this response was the result of a more efficient alveolar gas exchange in the acidic animals that enhanced the CO_2 expulsion and lowered arterial Pco_2.[30] Another explanation may be the reduction of CO_2 production and its release from severely hypoperfused tissues.

The CO_2 insufflation of the abdominal cavity of the normovolemic rats was well tolerated hemodynamically up to
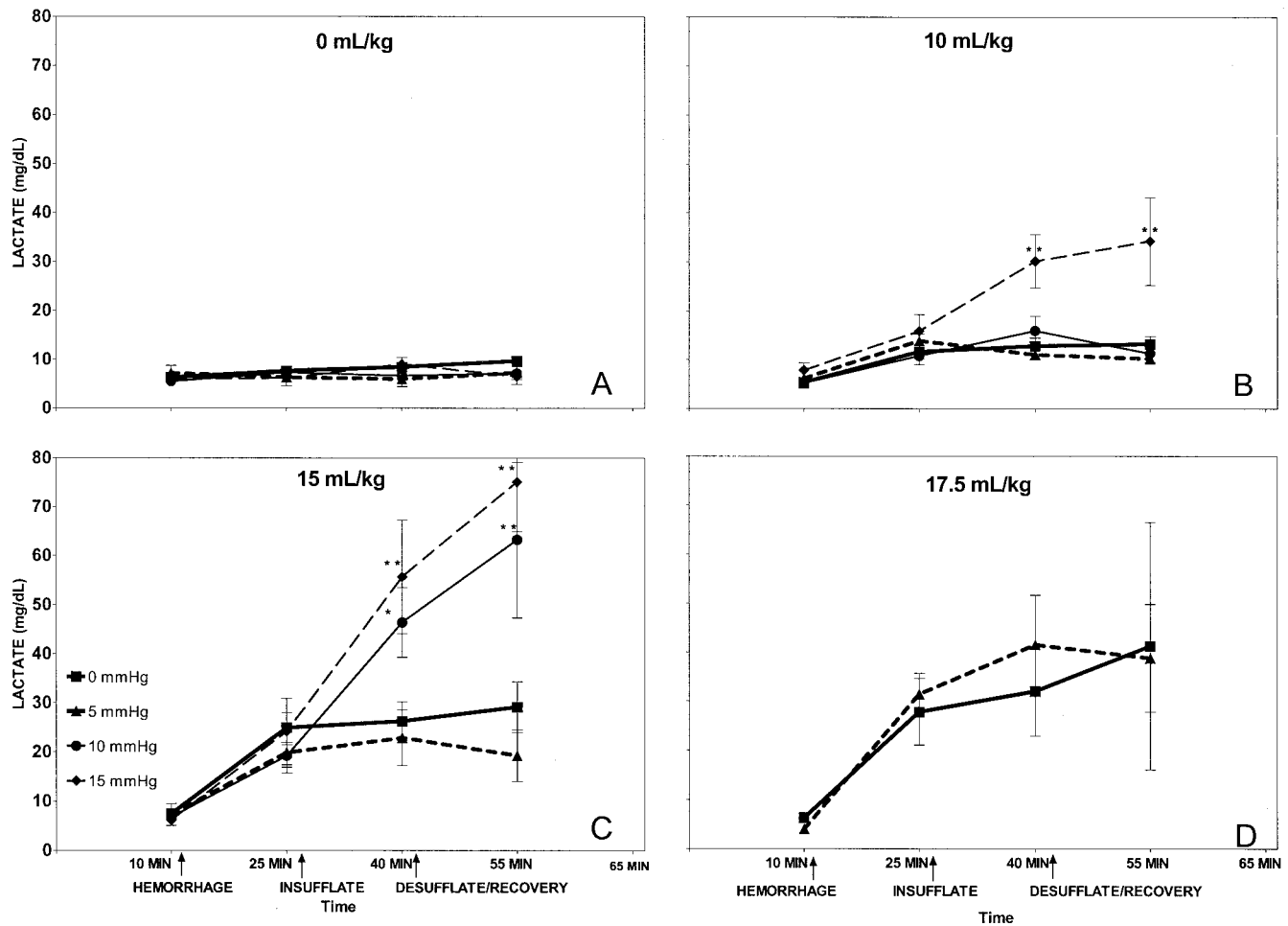
Fig. 8. *The lactate levels of the venous blood samples taken at 15-minute intervals during the experiment. The abdominal insufflation of normovolemic rats with CO_2, up to 15 mm Hg, did not change the lactate above the normal level (A). In the moderately bled rats (10 mL/kg), the 15 mm Hg insufflation pressure resulted in a significant rise of lactate levels ($p < 0.01$), whereas the lower insufflation pressures had little or no effect on the lactate concentrations (B). In the larger blood draw group (15 mL/kg), both 10 and 15 mm Hg PP pressures appeared to be excessive, causing significant accumulations of lactate in the venous bloods ($p < 0.05$ and $p < 0.01$, respectively [C]). The substantial rise of lactate levels in the 17.5-mL/kg groups is likely in response to severe blood loss rather than the PP procedure, since no difference was apparent between the insufflated groups and the controls (D).*

15 mm Hg and produced only minimal changes in mean arterial pressures. The most significant changes were the pressure rises that occurred after desufflation in the 10- and 15-mm Hg PP pressure groups. This response may be attributed to transient blood rush to the upper body of the rats as the PP pressure was released. This was apparent in the improvement of the ear microcirculation of the rats, returning to a normal pink color, once the abdomen was desufflated (personal observation). The reported rise of MAP in response to CO_2 PP was not seen in this model. This change in blood pressure is associated with the release of vasopressin in the healthy anesthetized subjects.[26,31–33] A significant acidosis developed 15 minutes after insufflation in these rats. This change was apparently the result of hypercapnia and a mild respiratory acidosis that developed despite mechanical ventilation. The intra-abdominal absorption of CO_2 may also

contribute in the increase of P_{CO_2}. The blood pH returned to normal after desufflation. No indication of metabolic acidosis was present. The respiratory compensation that effectively prevented the acidosis in hemorrhaged animals did not occur in normovolemic, insufflated animals. In another study, testing normovolemic rats, the optimum PP pressure for the laparoscopy procedure was suggested to be 10 mm Hg. Significant acidosis was reported to occur at higher PP pressures.[9] The suggested optimum pressure was determined on the spontaneously breathing rats that were insufflated for 10 minutes. In our study, where the rats were mechanically ventilated, 10 and 15 mm Hg PP pressure also produced significant acidosis. These changes were rapidly reversed after desufflation. In ventilated swine, the transperitoneal absorption of CO_2 was suggested to contribute to the development of hypercapnia and acidosis.[3]
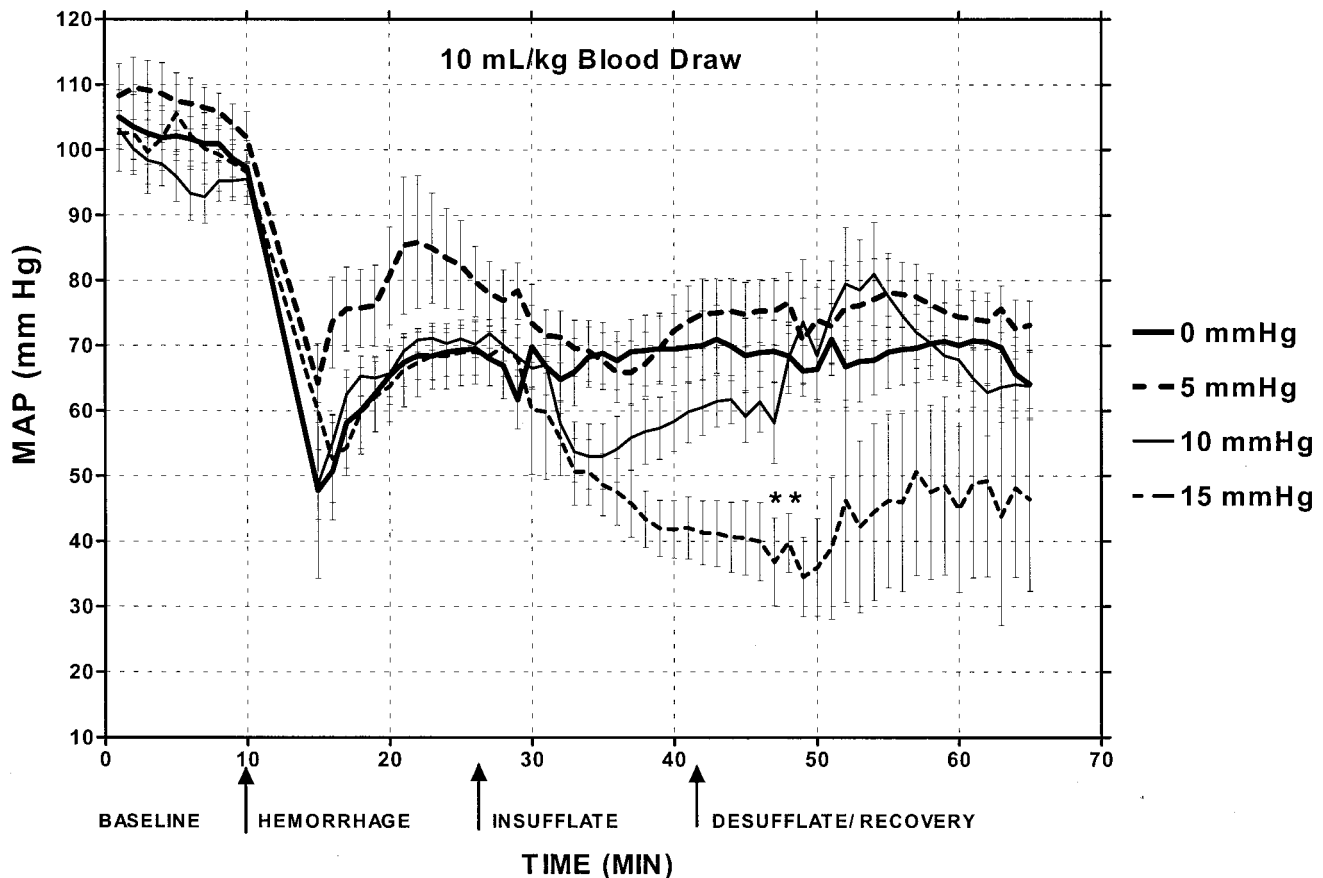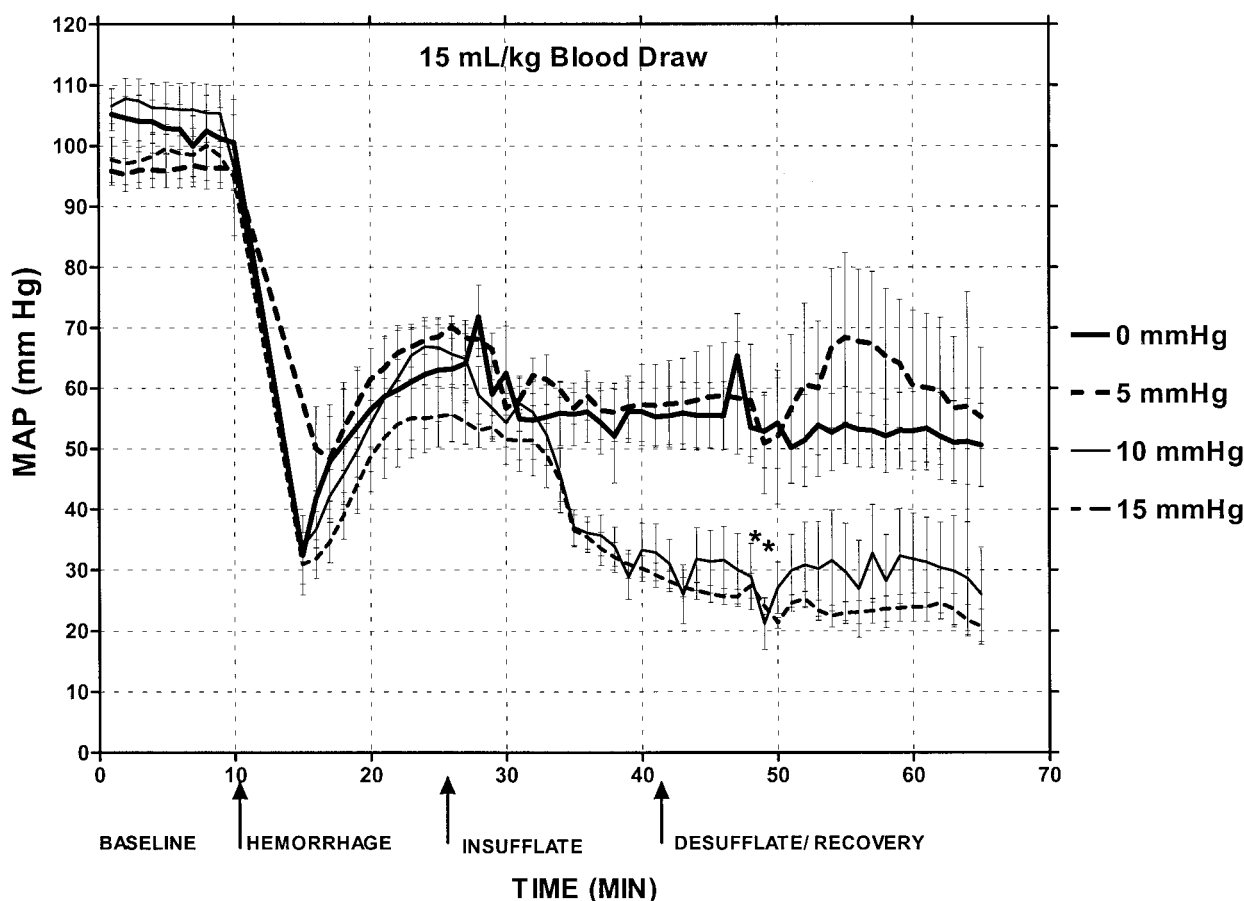
Fig. 9. *The averaged mean arterial pressures of hemorrhaged rats (10 mL/kg blood draw) as compared with those that were bled and insufflated with CO_2 at 5, 10, and 15 mm Hg pressure for 15 minutes. The 15 mm Hg PP pressure produced a significant and progressive reduction in MAPs (42%, p < 0.001), which were only partly reversed on desufflation.*

The increasing amount of blood loss reduced the safe limit of PP pressure from 15 mm Hg in control subjects to 10 mm Hg in moderately hemorrhaged rats (10 mL/kg) to 5 mm Hg in severely hemorrhaged (15 mL/kg) rats. PP pressure above these limits resulted in a significant decline of the mean arterial pressure, mild to severe acidosis, bicarbonate depletion, and a large increase of lactic acid in the venous blood. These changes were a clear indication of the development of a condition similar to abdominal compartment syndrome in which the abdominal pressure restricts the venous return and stroke volumes. This causes reduction of cardiac output, tissue perfusion, and oxygenation. The acidosis that developed in the more severely hemorrhaged (15 mL/kg) rats that were insufflated to 10 to 15 mm Hg became evident in the arterial blood samples that were collected after desufflation. This apparent contradiction may be a result of the complete blood compartmentalization between the upper and lower parts of the rat's body. During insufflation, whereas high levels of lactic acid were measured in the femoral venous blood samples (Fig. 8*C*), no change in arterial pH could be detected (Fig. 4*C*). This suggested that a large amount of lactic acid was produced but accumulated in the

lower extremities because of poor systemic circulation. Once the pressure was released and circulation was restored, the distribution of lactic acid throughout the body caused a significant reduction in the pH of the arterial blood samples (Fig. 4 *C*). The compensatory mechanisms to neutralize lactic acid were evident, as a significant reduction of bicarbonate and Pco_2 were measured in the blood. Lactic acid build-up is also reported in patients undergoing prolonged laparoscopic procedures.[33]

The hemodynamic effect of PP under various hypovolemic conditions was investigated as early as 1978.[27] In this study, dogs were anesthetized with pentobarbital injection and hemorrhaged at graded intervals (10% of the estimated blood volume each time), followed by CO_2 PP at 20 mm Hg. The result showed that PP produced only mild hemodynamic changes in the normovolemic dogs but caused increasingly more deterioration as the dogs were bled more severely. Additional cardiovascular depression was also observed when halothane was used as an anesthetic agent. In a more recent report, Steinman et al.[28] studied the PP effects in an uncontrolled hemorrhagic model in dogs. The CO_2 PP with an IAP of 10 mm Hg produced a significant decline in MAP,
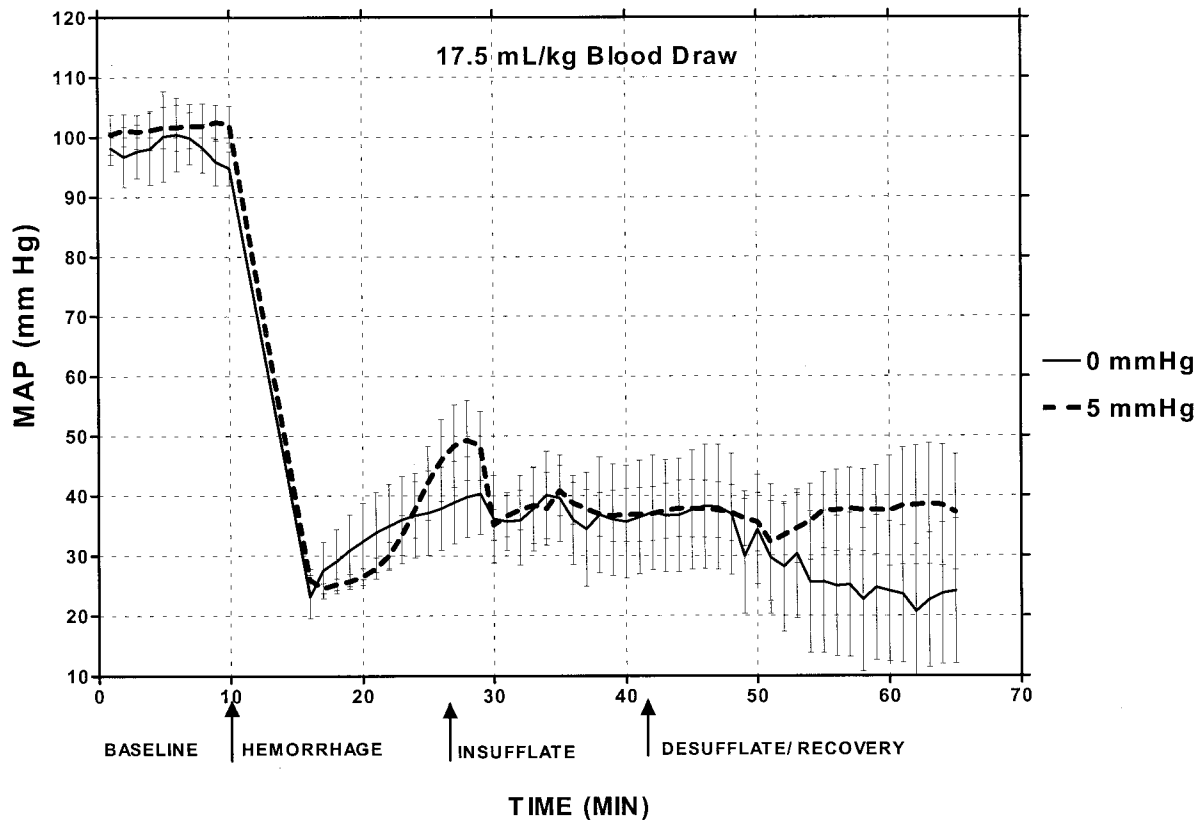
Fig. 10. *The averaged mean arterial pressures of hemorrhaged rats (15 mL/kg blood draw) as compared with those that were bled and insufflated with CO$_2$ at 5, 10, and 15 mm Hg for 15 minutes. Whereas the 5-mm Hg intra-abdominal pressure had a minimal impact, the 10- and 15-mm Hg PP pressures resulted in a progressive and irreversible decline in MAPs of the rats (p < 0.001).*

cardiac index, left ventricular stroke index, oxygen delivery, and oxygen consumption of the hypovolemic dogs with retroperitoneal hematoma. A similar PP (>12 mm Hg), however, caused no change in the hemodynamics of normovolemic animals. The reduction of cardiac index was because of diminished venous return secondary to extrinsic compression of the inferior vena cava and increased intrathoracic pressure. Using pigs as the animal model, Ho et al.[34] reported that 1-hour CO$_2$ PP at 15 mm Hg IAP resulted in a significant hypercapnia and acidemia, and a 20% reduction in stroke volume. Mild to moderate controlled hemorrhage, 10 to 20 mL/kg/h, exaggerated all these effects and reduced the stroke volumes to 75% and 55% of the controls. The resuscitation of the moderately hemorrhaged pigs (20 mL/kg/h) with 40 mL/kg of lactated Ringer's solution failed to prevent acidemia and the decline of the cardiac index. In contrast, in another porcine study, Grundel et al.[29] demonstrated that although the cardiac output and other hemodynamic parameters (e.g., MAP, systemic vascular resistance, and central venous pressure) as well as hepatic and renal blood flow were markedly reduced after hemorrhage (20 mL/kg/30 min), they returned nearly to their baseline levels after resuscitation with

a colloidal solution (6% hydroxyethyl starch, 20 mL/kg). The CO$_2$ PP at 12 mm Hg did not further depress the cardiovascular system or reduce hepatic and renal blood flow. The difference between the outcomes of these two studies points to a possible advantage of using colloidal over crystalloid solution for the resuscitation of the hypovolemic patient.

To our knowledge, this is the most systematic study that evaluates the hemodynamic effects of CO$_2$ PP at various pressures and under different hemorrhagic conditions. The goal of the study was to determine the safe limits of CO$_2$ PP under several defined hypovolemic conditions and use this information for development of a CO$_2$-driven, self-expanding hemostatic agent (FS foam) that is intended to be used to control internal bleeding in trauma patients.

Our results conclude that the safe limit of PP is dependent on the circulating blood volume. In the rat model, increasing amount of blood loss from 0% to 25% of the circulating blood volume reduces the safe level of CO$_2$ PP from 15 mm Hg to 5 mm Hg intra-abdominal pressures, respectively. The insufflation pressures above these limits lead to a condition similar to the abdominal compartment syndrome, with a significant and progressive decline in mean arterial pres-

Fig. 11. *The averaged mean arterial pressures of the hemorrhaged rats (17.5 mL/kg blood draw) as compared with those that were bled and insufflated with CO_2 at 5 mm Hg for 15 minutes. The severe drops in MAPs are in response to the loss of a large volume of blood. Additional PP procedure had no extra impact.*

sures and irreversible metabolic acidosis. The safe limits of PP recommended here are only valid in mechanically ventilated, anesthetized rats with a controlled hemorrhage. In spontaneously breathing animals with uncontrolled intra-abdominal bleeding, the safe limit of PP may vary and requires further investigation. Nevertheless, under the experimental conditions described here, the abdominal insufflation pressure up to 5 mm Hg appears to be safe and consistent with any degree of blood loss.

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